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NS5A inhibitors: A new breakthrough for the treatment of chronic hepatitis C

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COMMENTARY ON:

Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. Gao M, Nettles RE, Belema M, Snyder LB, Nguyen VN, Fridell RA, Serrano-Wu MH, Langley DR, Sun JH, O'Boyle DR 2nd, Lemm JA, Wang C, Knipe JO, Chien C, Colonno RJ, Grasela DM, Meanwell NA, Hamann LG. Nature. 2010;465(7294):96–100. Copyright (2010). Abstract reprinted with permission from Macmillan Publishers Ltd.

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Abstract: *The worldwide prevalence of chronic hepatitis C virus (HCV) infection is estimated to be approaching 200 million people. Current therapy relies upon a combination of pegylated interferon-alpha and ribavirin, a poorly tolerated regimen typically associated with less than 50% sustained virological response rate in those infected with genotype 1 virus. The development of direct-acting antiviral agents to treat HCV has focused predominantly on inhibitors of the viral enzymes NS3 protease and the RNA-dependent RNA polymerase NS5B. Here we describe the profile of BMS-790052, a small molecule inhibitor of the HCV NS5A protein that exhibits picomolar half-maximum effective concentrations (EC(50)) towards replicons expressing a broad range of HCV genotypes and the JFH-1 genotype 2a infectious virus in cell culture. In a phase I clinical trial in patients chronically infected with HCV, administration of a single 100-mg dose of BMS-790052 was associated with a 3.3 log(10) reduction in mean viral load measured 24 h post-dose that was sustained for an additional 120 h in two patients infected with genotype 1b virus. Genotypic analysis of samples taken at baseline, 24 and 144 h post-dose revealed that the major HCV variants observed had substitutions at amino-acid positions identified using the in vitro replicon system. These results provide the first clinical validation of an inhibitor of HCV NS5A, a protein with no known enzymatic function, as an approach to the suppression of virus replication that offers potential as part of a therapeutic regimen based on combinations of HCV inhibitors.*

Keywords: Boceprevir; Telaprevir; Pegylated interferon; Ribavirin; Protease inhibitors; Polymerase inhibitors; Interferon lambda; IL28B.

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Abbreviations: DAAs, direct acting antivirals; SVR, sustained virological response; RVR, rapid virological response; PEG-IFN, pegylated-interferon; RBV, ribavirin; HCC, hepatocellular carcinoma.

Recently, Gao et al. identified a potent HCV inhibitor targeting the non-traditional viral protein NS5A [1]. HCV is an enveloped virus with a 9.6 kb single-stranded RNA genome [2], a member of the Flaviviridae family, genus *Hepacivirus*. The HCV lifecycle begins with virion entry into the cytosol with a complex series of steps including attachment to its specific host cell receptor. The HCV RNA genome serves as a template for viral replication via a negative strand copy and as a messenger RNA for virus production. It is translated into a polyprotein that is cleaved by both host and viral proteases to release the individual enzymes and proteins that mediate virus replication, assembly, and release. All of the HCV enzymes are essential for HCV replication and are, therefore, potential targets for drug discovery. The knowledge of the structures of the NS3 protease and NS5B polymerase has allowed structure-based drug design, leading to the development of inhibitors of these enzymes. Several findings suggest that HCV modulation of IFN induction and signalling attenuates the expression of IFN-stimulated genes, allowing HCV to escape the antiviral actions of the host response [3].

The standard of care (SOC) is the association of pegylated interferon (PEG-IFN) plus ribavirin (RBV). Sustained virological response (SVR) defined by an absence of HCV RNA measured at 24 weeks after cessation of treatment and equivalent to viral eradication, is associated with a reduction in the risk of cirrhosis and HCC [4]. It has been recently reported that 12 weeks post-treatment follow-up is as relevant as 24 weeks to define SVR [5]. At present, in genotype 2 or 3 infected patients, SVR rates approach 80%; in genotype 1 SVR rates reach less than 50%. It has been shown that some interferon-stimulated genes are highly expressed in non-responders; thus, pre-activation of the IFN system in these patients appears to limit the effect of IFN antiviral therapy [6].

New drug therapies such as protease and polymerase inhibitors, designated as direct-acting antivirals (DAAs), are under development [7]. In HCV genotype 1 patients, very promising results have been reported when the protease inhibitors telaprevir or boceprevir are added to the current SOC. The final results of phase III studies have shown that they increase the SVR rates

from less than 50% (PEG-IFN plus RBV) to approximately 70% (PEG-IFN plus RBV plus protease inhibitor) [8–13]. The near future SOC will consist of a regimen that combines a protease inhibitor with PEG-IFN plus RBV.

A particularly interesting perspective would be IFN-sparing regimens that rely solely on DAA combinations. Therefore, the identification and development of new molecules with different mechanisms of action, having additive or synergistic effects, is needed. The goals for a DAA combination should be to increase antiviral efficacy, minimize the emergence of resistance, and limit side effects. The first study of a combination of DAAs in patients was the proof-of-concept INFORM-1 study [14]. In this randomised, placebo-controlled, double-blind trial, 87 genotype 1 infected patients were randomized to receive up to 13 days of either oral combination therapy with RG7227/danoprevir, a NS3/4A protease inhibitor, and RG7128, a nucleoside polymerase inhibitor, or with matched placebos. The median reduction in HCV-RNA from the baseline was 5 $\log_{10}$, which fell below the level of detection in 88% of the patients who received the highest dose of both danoprevir (900 mg bid) and RG7128 (1000 mg bid). No evidence of resistance to either compound was observed during the study and no serious adverse events were reported. The antiviral efficacy was similar in naïve and treatment-experienced patients, including non-responders. In the final cohort of patients who received the highest dose of RG7227 and RG7128, 100% achieved early virological response after 24 weeks of PEG-IFN and RBV treatment. The final SVR results will certainly be interesting.

There is a great need for additional HCV antivirals to provide more effective, better-tolerated treatment options that can be used in combination therapy, which will be necessary for building successful treatment regimens. In general, the development of antivirals has focused on targeting viral proteins with known enzymatic activities, such as protease or polymerase that are readily recapitulated *in vitro*. However, a major breakthrough

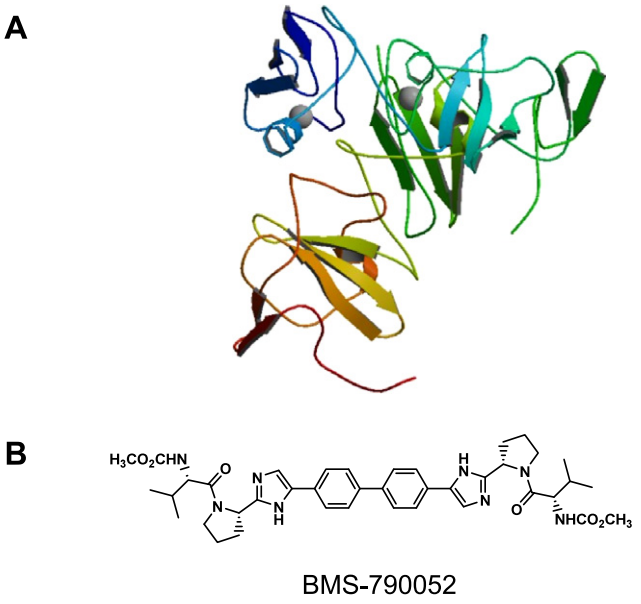

Fig. 1. The N terminus of NS5A (domain I) crystallized in alternative dimeric forms. (A) Structure of NS5A. (B) Structure of BMS-790052.

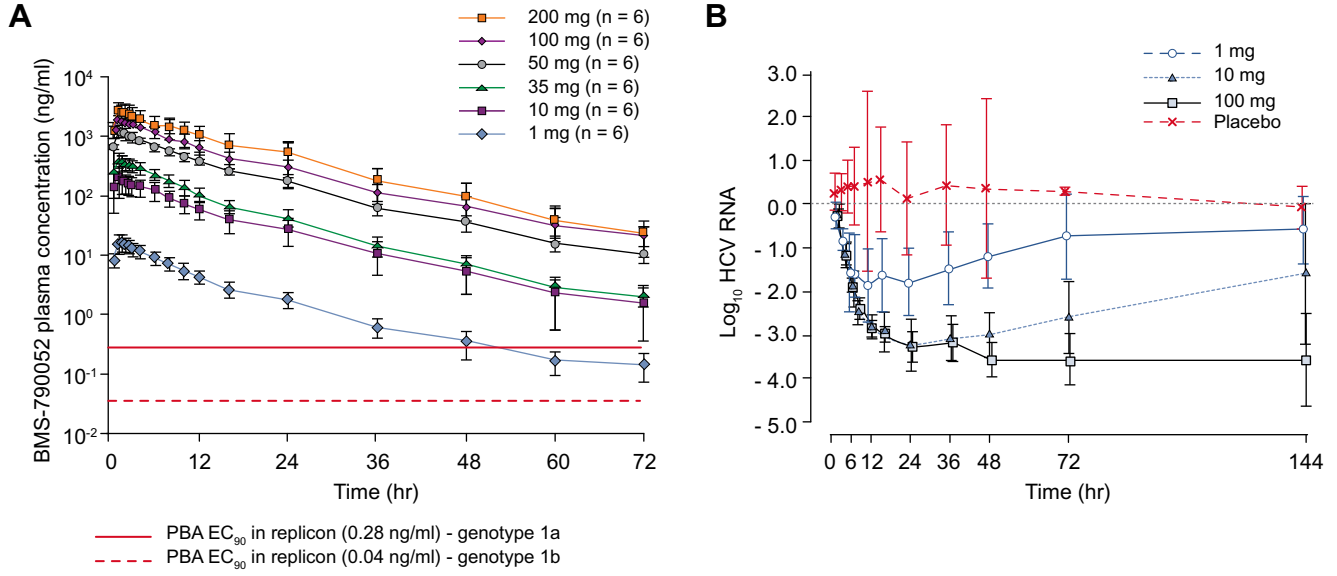

Fig. 2. Mean plasma concentration–time profile of BMS-790052. (A) Mean plasma concentration–time profile (time 0–72 h) of BMS-790052 after single oral administration of 1–200 mg of drug to healthy subjects. In a double-blind, placebo-controlled, sequential, single ascending-dose study, eight male or female subjects were randomized within each dose panel (1, 10, 25, 50, 100 and 200 mg) to drug or placebo in a ratio of 3:1. BMS-790052 or placebo was administered in the fasted state. The plasma samples obtained at various times were analysed for BMS-790052 by a validated liquid chromatography tandem mass spectrometry assay. Pharmacokinetic parameter values for individual subjects were derived by non-compartmental methods by a validated pharmacokinetic analysis programme. PBA-EC_{90} = protein-binding-adjusted EC_{90} for the individual genotype in a replicon assay. Error bars, standard deviation. From Ref. [1]. (B) Mean change in $\log_{10}$ HCV RNA with 90% confidence intervals after administration of single oral doses of BMS-790052 to HCV-infected patients. In a double-blind, placebo-controlled, sequential, single ascending-dose study, six subjects were randomized within each dose panel (1, 10, 100 mg) to drug or placebo in a ratio of 5:1. BMS-790052 or placebo was administered in the fasted state. Owing to a dosing error, all six subjects received BMS-790052 in the 1 mg panel. One subject in the 10 mg panel withdrew from the study 8 h after administration of the study drug for nondrug-related reasons; HCV RNA data from the subject are included up until the subject withdrew. From Ref. [1].

Table 1. Resistance profile of genotype 1a and 1b replicons exposed to BMS-790052 adapted from [18].

Amino acid substitution	EC_{50} (pM)[1]	Fold resistance	Replication level (%)
1b replicon			
Wild type	2.6 ± 0.3	1	100
L31F	12.6 ± 1.2	5	146 ± 44
L31V	61 ± 15	23	145 ± 44
P32L	44.7 ± 21	17	18 ± 6
Y93H	49.2 ± 12.8	19	20 ± 7
Y93N	73.5 ± 5.5	28	21 ± 6
1a replicon			
Wild type	0.006 ± 0.004	1	100
M28T	4.1 ± 0.4	683	31 ± 23
Q30H	8.7 ± 1.9	1,450	75 ± 31
Q30R	7.3 ± 1.1	1,217	41 ± 16
L31M	2.1 ± 0.6	350	55 ± 15
L31V	20.1 ± 6.0	3,350	117 ± 29
P32L	1.4 ± 0.2	233	18 ± 5
Y93C	11.1 ± 4.0	1,850	11 ± 7

[1]Mean ± standard deviation, determined in transient transfection assays ($n \geqslant 3$).

has recently been achieved that advances HCV inhibition beyond these traditional approaches. Researchers at Bristol-Myers Squibb have now reported that a compound, BMS-790052, with a new mechanism of action led to dramatic reductions in viral load and produced few side effects in a phase I clinical study [1]. BMS-790052 targets NS5A, a HCV non-structural protein that possesses no enzymatic activity but plays a critical role in regulating viral replication and host cell interactions. Structures of NS5A and BMS-790052 are provided in Figs. 1A and 1B. Although the function of HCV NS5A is still poorly understood, the BMS researchers have shown that small molecules targeting this protein are potent and specific inhibitors of viral replication.

NS5A is a membrane-associated phosphoprotein present in basally phosphorylated (p56) and hyperphosphorylated (p58) forms [15,16]. It was previously reported that only p58-defective mutants could be complemented in *trans*, and NS5A is involved in HCV virion production, suggesting that different forms of NS5A exert multiple functions at various stages of the viral life cycle. The N terminus of NS5A (domain I) has been crystallized in alternative dimeric forms and contains both zinc- and RNA-binding domains, properties that have been demonstrated *in vitro* [16] (Fig. 1). NS5A has been shown to interact with a number of host proteins and is implicated in interferon resistance *in vivo* [15]. The strategy used by Gao et al. to identify a lead HCV NS5A inhibitor provides a contemporary demonstration of the effectiveness of an approach to drug discovery based on chemical genetics [17]. Indeed, this methodology is uniquely applicable to targets similar to HCV NS5A for which precise functions are unknown and for which the development of biochemical assays are infeasible. The lead molecule was optimized into the clinical candidate BMS-790052 whose chemical structure is shown in Fig. 1B. This compound is active at picomolar concentrations *in vitro* towards replicons expressing a broad range of HCV genotypes and acts in an additive to synergistic fashion with interferon and other small molecule antiviral compounds. Mean plasma concentration–time profile of BMS-790052 after single oral administration to healthy subjects is shown in Fig. 2A. The effect of this compound on viral load in HCV-infected subjects following single oral doses of the compound are provided in Fig. 2B.

The resistance profile of BMS-790052 is shown in Table 1 which reveals that, inhibitor sensitivity maps to the N terminus of domain 1 of NS5A [1,18,19]. In addition, the BMS researchers demonstrated that NS5A inhibitors, as well as an active-site inhibitor that specifically binds NS3 protease, could block the hyperphosphorylation of NS5A, which is believed to play an essential role in the viral life cycle.

Phase II clinical studies combining BMS-790052 with the NS3 protease inhibitor BMS-650032 are ongoing and interim results have shown that this combination therapy alone or with PEG-IFN and RBV results in undetectable HCV RNA through 12 weeks of therapy in HCV genotype 1 null responders [20].

At present, several studies of DAA combinations are ongoing (Table 2) [20–22].

In conclusion, clinical proof-of-concept has recently been achieved with NS5A inhibitors, indicating that small molecules targeting a non-traditional viral protein without any known enzymatic activity can also have profound antiviral effects in HCV-infected subjects. Achieving high potency and selectivity against a non-mammalian target, the traditional goal of antiviral medicinal chemistry, has in the past translated into a wider therapeutic index in the clinic. Although preliminary, these data indicate that inhibitors of HCV NS5A offer considerable promise for the treatment of HCV infection. Once new DAAs become available, treatment strat-

Table 2. Some combinations under development in phase 2 clinical studies.

Molecules	Company
GS9256 (NS3/4A inhibitor) and GS9190 (non-nuc polymerase inhibitor)	Gilead [21]
BI201335 (NS3/4A inhibitor) and BI297127 (non-nuc polymerase inhibitor)	Boehringer [22]
BMS-650032 (NS3/4A inhibitor) and BMS-790052 (NS5a inhibitor)	BMS [20]
Telaprevir (NS3/4A inhibitor) with VX-222 (non-nuc polymerase inhibitor)	Vertex
RG7227 (NS3/4A inhibitor)/Ritonovir and RG7128 (Nuc polymerase inhibitor)	Roche
IDX320 (NS3/4A inhibitor) and IDX184 (Nuc polymerase inhibitor)	Idenix
ABT-450 (NS3/4A inhibitor)/Ritonovir and ABT-072 (non-nuc polymerase inhibitor)	Abbott

egies that combine several drugs with different mechanisms of action could hopefully result in IFN- and/or RBV-sparing regimens. Ongoing studies are directed towards demonstrating that such combinations of DAAs have synergistic antiviral potency, a low risk of resistance with a good safety profile.

Conflict of interest

The authors who have taken part in this study have declared a relationship with the manufacturers of the drugs involved. Tarik Asselah has been an investigator or speaker for BMS, Gilead, Merck, Roche, and Tibotec.

References

[1] Gao M, Nettles RE, Belema M, Snyder LB, Nguyen VN, Fridell RA, et al. Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. Nature 2010;465:96–100.

[2] Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989;244:359–362.

[3] Asselah T, Bièche I, Sabbagh A, Bedossa P, Moreau R, Valla D, et al. Gene expression and hepatitis C virus infection. Gut 2009;58:846–858.

[4] Maylin S, Martinot-Peignoux M, Moucari R, Boyer N, Ripault MP, Cazals-Hatem D, et al. Eradication of hepatitis C virus in patients successfully treated for chronic hepatitis C. Gastroenterology 2008;135:821–829.

[5] Martinot-Peignoux M, Stern C, Maylin S, Ripault MP, Boyer N, Leclere L, et al. Twelve weeks posttreatment follow-up is as relevant as 24 weeks to determine the sustained virologic response in patients with hepatitis C virus receiving pegylated interferon and ribavirin. Hepatology 2010;51: 1122–1126.

[6] Asselah T, Bieche I, Narguet S, Sabbagh A, Laurendeau I, Ripault MP, et al. Liver gene expression signature to predict response to pegylated interferon plus ribavirin combination therapy in patients with chronic hepatitis C. Gut 2008;57:516–524.

[7] Asselah T, Benhamou Y, Marcellin P. Protease and polymerase inhibitors for the treatment of hepatitis C. Liver Int 2009;29:57–67.

[8] Sherman KE, Flamm SL, Afdhal NH, Nelson DR, Sulkowski MS, Everson GT, et al. Telaprevir in combination with peginterferon Alfa2a and ribavirin for 24 or 48 weeks in treatment-naïve genotype 1 HCV patients who achieved an extended rapid viral response: final results of Phase 3 ILLUMINATE study. Hepatology 52, AASLD 2010; Abstract LB-2.

[9] Jacobson IM, McHutchison JG, Dusheiko GM, Di Bisceglie AM, Reddy R, Bzowej NH, et al. Telaprevir in combination with peginterferon and ribavirin in genotype 1 HCV treatment-naïve patients: final results of Phase 3 ADVANCE study. Hepatology 52, AASLD 2010; Abstract 211.

[10] McHutchison JG, Manns MP, Muir AJ, Terrault NA, Jacobson IM, Afdhal NH, et al. Telaprevir for previously treated chronic HCV infection. N Engl J Med 2010;362:1292–1303.

[11] Kwo PY, Lawitz EJ, McCone J, Schiff ER, Vierling JM, Pound D, et al. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naive patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. Lancet 2010;376:705–716.

[12] Poordad F, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, et al. Boceprevir combined with peginterferon alfa-2b/ribavirin for treatment-naive patients with hepatitis C virus genotype 1: SPRINT-2 final results. Hepatology 52, AASLD 2010; Abstract LB-4.

[13] Bacon BR, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, et al. HCV RESPOND-2 final results: high sustained virological response among genotype 1 previous non-responders and relapsers to peginterferon/ribavirin when re-treated with boceprevir plus pegintron (peginterferon alfa-2b)/ ribavirin. Hepatology 52, AASLD 2010; Abstract 216.

[14] Gane E, Roberts S, Stedman C, Angus PW, Ritchie B, Elston R, et al. Oral combination therapy with a nucleoside polymerase inhibitor (RG7128) and danoprevir for chronic hepatitis C genotype 1 infection (INFORM-1): a randomised, double-blind, placebo-controlled, dose-escalation trial. Lancet 2010;376:1467–1475.

[15] Holler TP, Parkinson T, Pryde DC. Targeting the non-structural proteins of hepatitis C virus: beyond hepatitis C virus protease and polymerase. Expert Opin Drug Discov 2009;4:3.

[16] Love RA, Brodsky O, Hickey MJ, Wells PA, Cronin CN. Crystal structure of a novel dimeric form of NS5A domain I protein from hepatitis C virus. J Virol 2009;83:4395–4403.

[17] Stockwell BR. Exploring biology with small organic molecules. Nature 2004;432:846–854.

[18] Lemm JA, O'Boyle 2nd D, Liu M, Nower PT, Colonno R, Deshpande MS, et al. Identification of hepatitis C virus NS5A inhibitors. J Virol 2010;84: 482–491.

[19] Fridell RA, Qiu D, Wang C, Valera L, Gao M. Resistance analysis of the hepatitis C virus NS5A inhibitor BMS-790052 in an in vitro replicon system. Antimicrob Agents Chemother 2010;54:3641–3650.

[20] Lok AS, Gardiner DF, Lawitz E, Martorell C, Everson GT, Ghalib RH, et al. Combination therapy with BMS-790052 and BMS-650032 alone or with pegIFN/RBV results in undetectable HCV RNA through 12 weeks of therapy in HCV genotype 1 null responders. Hepatology 52, AASLD 2010; Abstract LB-8.

[21] Zeuzem S, Buggisch P, Agarwal K, Manns MP, Marcellin P, Foster G, et al. Dual, triple, and quadruple combination treatment with a protease inhibitor (GS-9256) and a polymerase inhibitor (GS-9190) alone and in combination with ribavirin (RBV) or PegIFN/RBV for up to 28 days in treatment naïve, genotype 1 HCV subjects. Hepatology 52, AASLD 2010; Abstract LB-1.

[22] Zeuzem S, Asselah T, Angus PW, Zarski JP, Larrey D, Mullhaupt B, et al. Strong antiviral activity and safety of IFN-sparing treatment with the protease inhibitor BI 201335, the HCV polymerase inhibitor BI 207127 and ribavirin in patients with chronic hepatitis C. Hepatology 52, AASLD 2010; Abstract LB-7.